Identification and isolation of soluble histones from bovine milk and serum

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An immunoassay for soluble histones as trace components of biological fluids was developed on the basis of the dual capacity of histones to bind solid-phase DNA and monoclonal anti-histone antibody. Application of this histone-capture assay to bovine milk resulted in a positive signal, and DNA–cellulose chromatography was used to isolate histone-like material in microgram quantities. Western-blot analysis using a panel of anti-histone antibodies demonstrated the presence of histones H2A, H2B and H4 in apparently intact form. DNAase digestion experiments indicated that at least a portion of milk histone was complexed to DNA. Bovine serum was analysed in the same manner on serial DNA–cellulose columns, and H4 and partially degraded H2A were detected by Western-blot analysis. The finding of soluble histones in bovine milk and serum may account for unexpected results when these biological fluids are used as blocking reagents in Western blots and other immunoassays and may have ramifications in the origin and significance of anti-histone antibodies in human disease.

INTRODUCTION

Macromolecules normally found within cells have also been reported in other compartments *in vivo*. DNA was detected in human serum (Tan *et al.*, 1966) at concentrations ranging from 0.004 to 0.4 μg/ml (McCoubrey-Hoyer *et al.*, 1984) and in the μg/ml range in some patients with systemic lupus erythematosus (Raptis & Menard, 1980). Naked DNA has a half-life of less than 2 min in mice (Emlen & Mannik, 1980), but the stability of endogenous DNA in the circulation may be longer. Protection against nucleolytic degradation of serum DNA may be afforded by non-covalently bound proteins, and histones would be natural candidates. However, there is little evidence for the presence of histones *in vivo* outside the cell nucleus.

Soluble DNA is secreted by lymphocytes activated with phytohaemagglutinin (Rogers *et al.*, 1972), and a portion of lymphocyte membrane DNA is associated with histone-like proteins (Rogers & Kawahara, 1981). Cell-membrane-associated nucleohistone or DNA has been confirmed by numerous studies (Rekvig & Hannestad, 1980; Horneland *et al.*, 1983; Jacob *et al.*, 1984; Holers & Kotzin, 1985; Bennett *et al.*, 1985), and nucleohistone-like material was detected in tissue-culture media of mouse spleen cells (Atkinson *et al.*, 1985) and neural retinal cells (Shubert & LaCorbiere, 1985). It appears likely, therefore, that nucleohistones and DNA may not be uncommon in extracellular fluids, raising the possibility that soluble histones may exist in the circulation.

In addition to being of fundamental interest, histones in certain biological fluids may have practical ramifications. Bovine serum or non-fat dry milk have come into increasing use as protein carriers or blocking agents in solid-phase immunoassays for autoantibodies (Johnson *et al.*, 1984). Discrepancies in the literature on anti-histone-antibody specificities led to the suspicion that the blocking medium may affect the signal in these assays. Previous studies demonstrating DNA in serum, and the possibility that this DNA may be complexed with histones, suggested that soluble histones may account for these artefacts. For these reasons a study was undertaken to search for histone-like material in bovine serum and milk.

MATERIALS AND METHODS

Culture assay for soluble histones

Calf thymus DNA (Calbiochem–Behring, La Jolla, CA, U.S.A.) was diluted in PBS (0.01 M-sodium phosphate buffer/0.14 M-NaCl, pH 7.2) to a concentration of 2.5 μg/ml, followed by boiling for 15 min and immediate cooling in an ice bath. After overnight incubation in Immulon II microtitre plates (Dynatech Laboratories, Alexandria, VA, U.S.A.), the DNA solution was decanted, and 0.25 ml of gelatin (Baker) at 1 mg/ml was incubated in the wells for at least 18 h. Solutions to be tested for histone activity were incubated in DNA-coated wells for 2 h at room temperature with agitation. An IgG2a mouse monoclonal anti-histone antibody reactive with histones H2A, H2B and H2A–H2B dimers (antibody 'b'; Table 1) was then used to detect DNA-captured histones. After incubation for 1.5 h, bound anti-histone antibody was detected with peroxidase-conjugated goat anti-mouse IgG (Tago, Burlingame, CA, U.S.A.) according to e.l.i.s.a. methodology previously described (Rubin *et al.*, 1983). Total histone (Calbiochem–Behring) and polylysine (Miles Laboratories, Elkhart, IN, U.S.A.) were employed as controls in this assay.

DNA–cellulose affinity chromatography

Non-fat dry milk (Von's Grocery Co., Los Angeles, CA, U.S.A.) was dissolved in PBS at 150 mg/ml, and

Abbreviations used: e.l.i.s.a., enzyme-linked immunosorbent assay; FBS, fetal-bovine serum; H1, H2 etc., histone H1, H2 etc.

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Table 1. Individual-histone-binding specificities of monoclonal anti-histone antibodies

Individual histones were purified from calf thymus as previously described (Rubin, 1986*b*), and their reactivities with murine monoclonal anti-histone antibodies were determined by e.l.i.s.a.

Monoclonal antibody	Antibody binding to histone/polypeptide (*A*)							
	H1	H2A	H2B	H3	H4	H2A–H2B	Polylysine	Polyarginine
a	0.03	3.74	0.03	0.14	4.15	2.57	0.05	0.05
b	0.01	0.07	0.04	0.03	0.06	1.82	0.01	0.03
c	0.06	0.12	5.26	0.07	1.25	4.55	0.06	0.02

whey was prepared from this solution by ultracentrifugation in the SW 41 rotor (*r* 11 cm) of a Beckman centrifuge at 39000 rev./min for 1 h at 4 °C (Lonnerdal *et al.*, 1985).

DNA–cellulose was prepared by the method of Biogioni *et al.* (1978). Briefly, cellulose (Cellex N-1; Bio-Rad Laboratories, Richmond, CA, U.S.A.) was activated with cyanuric chloride (Sigma Chemical Co., St. Louis, MO, U.S.A.), which had been purified by extraction with chloroform. Dichlorotriazinyl–cellulose (activated cellulose) was suspended at a concentration of 1 g/4 ml of calf thymus DNA solution (0.5 mg/ml) with stirring at 4 °C. Spectrophotometric analysis showed that more than 80% of the DNA was bound to cellulose.

A 5 ml portion of settled DNA–cellulose containing 2.7 mg of DNA was suspended in either 800 ml of whey or 1000 ml of FBS (Irvine Scientific, Santa Ana, CA, U.S.A.), and incubated overnight at 4 °C with gentle stirring. The DNA–cellulose was collected by centrifugation at 3000 rev./min (1100 *g*) for 20 min at 4 °C and transferred to a 9 mm-diameter glass column. After washing with 500 ml of PBS at a rate of 0.5 ml/min, the column matrix was treated with 0.1 M-HCl, and 1 ml fractions were collected at a flow rate of 0.14 ml/min. Each fraction was evaluated by its A_{230}, was neutralized with 0.01 M-Tris/NaOH, and tested in the capture assay for soluble histone. Histone-positive fractions were pooled, dialysed against 0.05 M-acetic acid and freeze-dried. Protein determination on the water-soluble material was by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. As a control, DNA-cellulose to which no biological fluid was applied was subjected to the same washing and elution procedure; no protein or histone activity was detected.

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis on a 15% (w/v)-acrylamide slab gel with a 6% -acrylamide stacking gel was performed by the method of Laemmli (1970). Disulphide bonds in the samples were reduced by boiling for 5 min in 0.0625 M-Tris buffer, pH 6.8, containing 2.3% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 10% (v/v) glycerol. Samples applied to the gel (180 mm × 150 mm × 1.5 mm) were electrophoresed at 10 mA for 14 h and were stained with 0.05% Coomassie Blue R250 in methanol/acetic acid/water (9:2:9, by vol.) for 3 h.

Western-blot analysis

After SDS/polyacrylamide-gel electrophoresis the samples were electrophoretically transferred to nitrocellulose by the method of Towbin *et al.* (1979). Transfer took place at 260 mA for 3 h at 5 °C. The nitrocellulose was then cut into strips, which were subsequently soaked in 0.25% (w/v) gelatin (Baker, Phillipsburg, NJ, U.S.A.) in PBS for 2 h at room temperature to block non-specific binding sites. A set of monoclonal antibodies or human serum with anti-histone activity (described below), diluted with 0.25% gelatin in PBS/0.05% -Tween-20, pH 7.4, were allowed to react with the nitrocellulose strips for 1 h at room temperature. The strips were then washed by agitation in two changes of PBS/Tween for 1.5 h. Bound antibodies were detected by reaction with either goat anti-(mouse κ-chain) (Southern Biotechnology Associates, Birmingham, AL, U.S.A.) for the monoclonal antibodies, or goat anti-human IgG (Tago, Burlingame, CA, U.S.A.) for the human antibodies. The detecting antibodies were radiolabelled with ^{125}I (McConahey & Dixon, 1980) and diluted in 0.25% gelatin in PBS/Tween to 2×10^5 c.p.m./ml. After extensive washing with PBS/Tween for 2.0 h, the strips were dried and transferred to a cassette fitted with enhancing screen and Kodak X-Omat film for autoradiographic exposure for approx. 24 h at −70 °C.

Mouse monoclonal antibodies and human sera used for Western-blot analysis

The mouse monoclonal anti-histone and anti-DNA antibodies were derived from autoimmune mice by hybridoma technology. Anti-histone antibody 'b' and the anti-DNA antibody were obtained from an NZB/NZW mouse as previously described (Kotzin *et al.*, 1984). Monoclonal anti-histone antibodies 'a' and 'c' were derived from MRL/lpr/lpr mice. As shown in Table 1 and Fig. 1, antibody 'a', an IgG3, showed predominant reactivity with histones H2A and H4. Antibody 'c' was of the IgG2b isotype and was essentially monospecific for H2B. The specificity of the monoclonal anti-histone antibodies evaluated by e.l.i.s.a. using purified individual histones (Table 1) largely agreed with their reactivity in Western-blot format using total histones separated by SDS/polyacrylamide-gel electrophoresis (Fig. 1). Antibody 'b', which showed predominant reactivity by e.l.i.s.a. with the H2A–H2B complex, by Western blot detects H1, H2A, H2B and a smear in the H2A/H2B region of the gel. The smear is due to cross-contamination of H2A and H2B during electrophoretic transfer, resulting in H2A–H2B-complex formation on the nitrocellulose (Portanova *et al.*, 1986). Human sera with anti-histone antibodies were obtained from patients with procainamide-induced lupus (Rubin *et al.*, 1985).

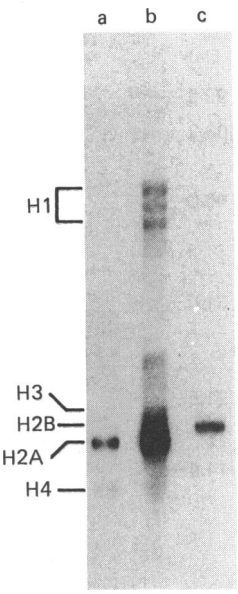

Fig. 1. Western blot of monoclonal anti-histone antibodies

Monoclonal antibodies 'a', 'b' and 'c' were allowed to react with total calf thymus histones after separation by SDS/polyacrylamide-gel electrophoresis and transfer to nitrocellulose.

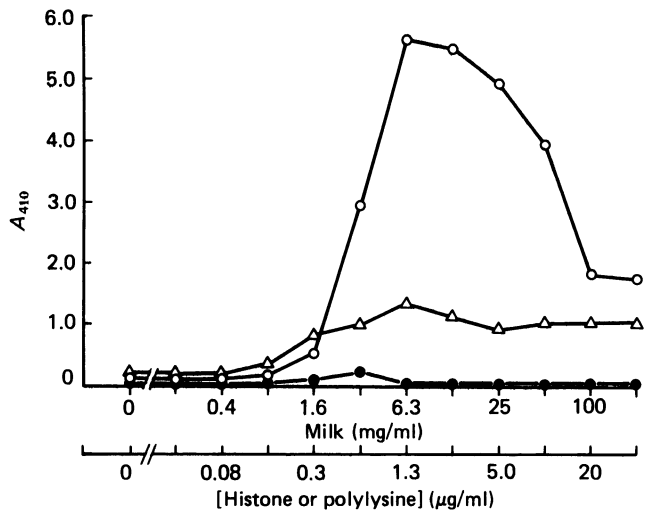

Fig. 2. Histone capture assay

Increasing concentrations of total histones (○), polylysine (●) or milk (△) were added to microtitre wells coated with denatured DNA. Histone binding was detected by an indirect e.l.i.s.a. using a mouse monoclonal anti-histone antibody.

DNAase I treatment

Samples were subjected to DNAase treatment in a solvent consisting of 0.25 mM-$CaCl_2$/1 mM-$MgCl_2$/0.5 mM-phenylmethanesulphonyl fluoride (Calbiochem–Behring) in PBS. DNAase I (Sigma) was added at 0.2 unit/ml and incubated for 4 h at 37 °C, followed by addition of EDTA to 4 mM to stop the reaction.

RESULTS

Detection of histone activity in milk

It was anticipated that, if histones were a component of milk, they would be present in low concentrations and detection would require an assay of high sensitivity and specificity. This was achieved by concentrating the putative histone components by their natural tendency to bind to DNA in a histone 'capture' assay. The assay format consisted of DNA immobilized to polystyrene, followed by incubation with the test material and detection of bound histone with a monoclonal anti-histone antibody.

As shown in Fig. 2, a total-histone concentration of approx. 0.3 μg/ml was detectable, with the signal increasing linearly up to 1.3 μg/ml. Above this concentration, histone detection decreased. Polylysine, a basic DNA-binding polypeptide which does not react with the monoclonal anti-histone antibody (Table 1), did not show significant activity. When milk solution was applied to this assay, histone reactivity was initially detectable at a milk-solids concentration of 1 mg/ml, followed by an approximately linear increase and plateau at 6 mg/ml. This binding curve resembled the histone dose–response curve, although displaying approx. 4-fold less signal at maximum response. In contrast, up to 10% (w/v) milk applied to wells devoid of DNA did not produce histone activity (results not shown), indicating that the histone-like material in milk bound to the DNA component of the plate.

Isolation of histone activity from milk

The histone-capture-assay results suggested that DNA–cellulose affinity chromatography may be a promising methodology for isolating putative histones from milk. However, flow problems of concentrated milk solution (presumably due to casein micelles) hampered this method, but the whey component prepared by ultracentrifugation surmounted this problem. As shown in Fig. 3, the whey obtained from ultracentrifugation (U)

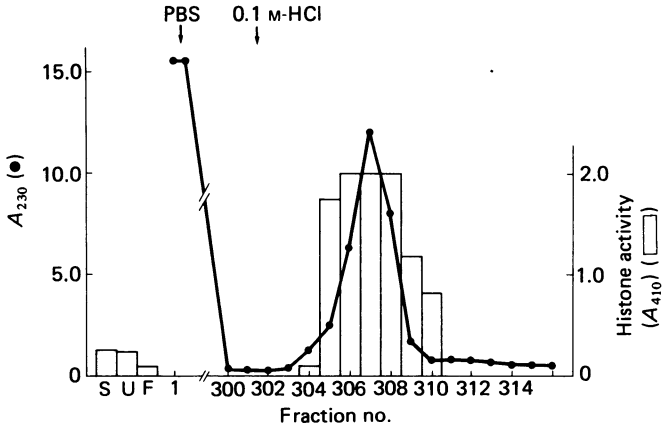

Fig. 3. Isolation of histone activity from milk by DNA–cellulose affinity chromatography

The starting milk solution (S) was ultracentrifuged (U), and 800 ml of whey was applied to the DNA–cellulose column. After the initial flow-through (F), the column was extensively washed with PBS, followed by elution of protein with 0.1 M-HCl. Histone activity (□) and protein concentration (●) were measured in fractions after appropriate dilution.

gave the same histone signal as the starting milk solution (S), suggesting that putative histone was not associated with insoluble aggregates. Histone activity of the flow-through material (F) from the DNA–cellulose column was decreased, indicating adsorption of histone activity on to the column. After extensive washing with PBS, the column was subjected to elution with 0.1 M-HCl. A single large peak was detected at the apparent void volume. A good correspondence between A_{230} (protein concentration) and A_{410} (histone activity in the capture immunoassay) was observed. Histone-positive fractions were pooled, dialysed and freeze-dried before subsequent analysis.

This isolation procedure was performed three separate times. Starting with 15 g of milk protein, the maximum yield of DNA-binding proteins was 4.1 mg, which contained 375 μg of histone activity by the histone-capture assay.

Characterization of DNA-binding proteins from milk

Spectrophotometric analysis of material from the DNA–cellulose column (Fig. 4) showed an absorption maximum at 273 nm and an A_{280}/A_{230} ratio of 0.20. No peak or shoulder was observed at 260 nm, indicating the absence of significant amount of DNA in the preparation. This spectrum can be compared with that for total histones, which have an absorption maximum at 274 nm and an A_{280}/A_{230} ratio of 0.13. It appears likely, therefore, that the isolated material has a relatively low aromatic-amino-acid content, approximately intermediate between that of histones and a protein such as albumin.

By using SDS/15%-polyacrylamide-gel electrophoresis the isolated DNA-binding proteins produced three dominant bands at positions corresponding to

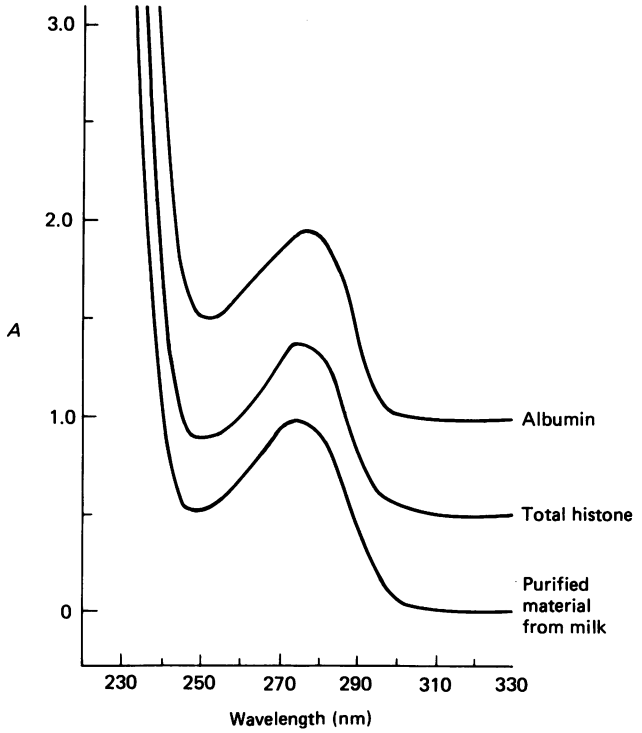

Fig. 4. Absorption spectrum of DNA-binding proteins from milk: comparison with those of authentic histones and albumin

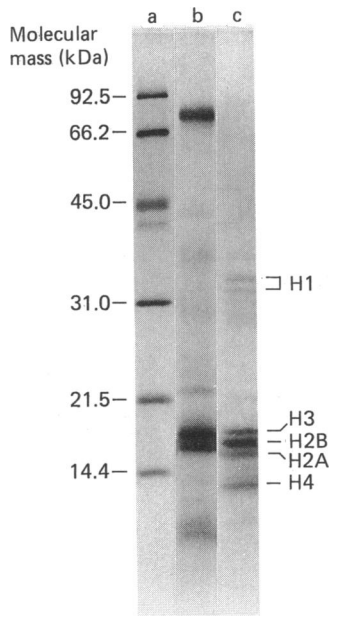

Fig. 5. SDS/polyacrylamide-gel electrophoresis of proteins isolated from milk (lane b)

Molecular masses of marker proteins (lane a) and total calf thymus histones (lane c) are shown for comparison. The 18 kDa and 16 kDa bands in the milk-derived material had positions similar to, but not identical with, those of histones H3, H2B and/or H2A.

78 kDa, 18 kDa and 16 kDa, as well as numerous faint bands throughout the gel (Fig. 5). By comparison with total histones run on a parallel gel, the 18 kDa and 16 kDa bands were similar to, although not identical with, histones H3, H2B and/or H2A, but no definite bands corresponding to H1 or H4 were observed. However, when Western-blot analysis (Fig. 6) was used, binding of monoclonal anti-histone antibodies specific for H2A, H4 and H2B was demonstrated. Unlike the position of the bands in the Coomassie Blue-stained gels (Fig. 5), the monoclonal antibodies marked positions on the blot which corresponded precisely to the electrophoretic position of authentic histones. No other bands were detected, including bands corresponding to the positions of H1 and H3. These results indicate that at least some histones were present in bovine milk in apparently undegraded form. In addition, other unidentified non-histone DNA-binding proteins were present.

Evidence for the co-presence of DNA in milk

Histone in milk was readily detected by exploiting its capacity to bind to DNA in solid-phase assay (Fig. 2). The reciprocal assay, detection of DNA by virtue of its capacity to interact with solid-phase histone, was also demonstrable by using a monoclonal anti-DNA antibody (results not shown). When whey from milk was applied to solid-phase histone and probed with anti-DNA antibody, a strong signal was obtained (Table 2). The presence of DNA in whey was confirmed by elimination of anti-DNA antibody binding by pre-digestion with DNAase. Interestingly, when a replicate sample was probed with a monoclonal anti-histone antibody, enhanced antibody binding was also detected, and this was decreased 3-fold by DNAase pretreatment. These results suggest that DNA is present in whey and that at

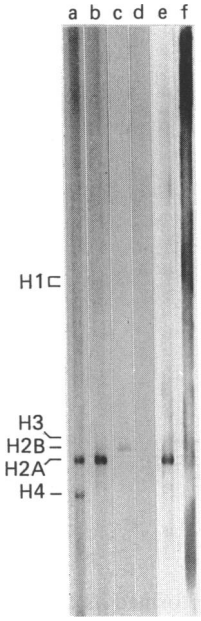

Fig. 6. Western blot of isolated proteins from milk

DNA-binding proteins were separated by SDS/polyacrylamide-gel electrophoresis, electrophoretically transferred to nitrocellulose and allowed to react with the following antibodies: a, mouse monoclonal anti-H2A/anti-H4; b, mouse monoclonal anti-(H2A/H2B); c, mouse monoclonal anti-H2B; d, normal mouse serum; e, human polyclonal anti-(H2A/H2B); f, normal human serum. The position of the bands on these blots corresponded to the positions of authentic histones (not shown in this immunoblot).

least some of this DNA exists as a complex with histones. Total histone did not bind anti-DNA antibody or bind anti-histone antibody in a DNAase-sensitive manner. DNA-binding material from milk behaved in a similar way, indicating absence of DNA from the purified material.

Isolation of histone-like materials from FBS

Preliminary studies using Western-blot analysis suggested that FBS may also contain histone-like material (Waga *et al.*, 1986), although FBS displayed only marginal activity in the histone-capture assay. Initial attempts to isolate histone from FBS by DNA–cellulose chromatography were largely unsuccessful, although a large number of non-histone bands were detected by SDS/polyacrylamide-gel electrophoresis. However, when the material which did not bind to DNA–cellulose was re-applied to a second DNA–cellulose column, substantially greater yields of histone activity were obtained. Typical results of this sequential DNA–cellulose chromatography are shown in Fig. 7. The flow-through material from column 1 (F1) when applied to a second column displayed high histone content in the HCl eluate. In addition, residual histone activity in the column-2 flow-through material (F2) was detected and could be recovered on a third, and subsequently a fourth, DNA–cellulose column. The total amounts of protein and histone activity recovered from the four columns were 1.4 mg and 38 μg respectively.

When subjected to SDS/polyacrylamide-gel electrophoresis, 13–14 major bands were detectable, with another 7–8 minor bands ranging in apparent molecular mass from under 10 kDa to over 100 kDa (Fig. 8, lane b). Multiple bands in the region of the gel corresponding to H3, H2B and H2A (lane a) were discernible in the material isolated from both FBS (lane b) and milk (lane c). However, most of the proteins comprising the FBS isolate were substantially different from those in the milk isolate.

Despite the lack of a clear histone profile in the FBS isolate by Coomassie Blue staining, Western-blot analysis showed definite reaction of discrete bands with monoclonal anti-histone antibodies. The monoclonal antibody reactive with H4 and H2A stained bands weakly at the positions corresponding to these histones (Fig. 9, lane 1a). However, the monoclonal antibody to H2B was non-reactive (lane 1c). Monoclonal antibody 'b' showed weak reaction with H2A as well as strong staining on a band migrating slightly slower than H4 (lane 1b). Evidence that this band was a degradation product of H2A and/or H2B was suggested by the observation that calf thymus chromatin, partially degraded by endogenous proteinases (Watson & Moudrianakis, 1982) also displayed a band at this position which bound the monoclonal antibody (lane 2b). No

Table 2. DNA-histone complexes in milk: effect of DNAase pre-treatment on histone-binding capacity

A portion of the material to be assayed was treated with DNAase as described in the Materials and methods section. A (200 μl) portion of this or the untreated preparation was incubated in histone-coated wells. Either monoclonal anti-histone antibody 'b' or anti-DNA antibody was subsequently added and binding detected by e.l.i.s.a. The absorbance data shown were obtained after subtracting the background binding of the monoclonal anti-DNA antibody (0.02 *A*) or the anti-histone antibody (0.18 *A*).

Material applied	[Protein] (mg/ml)	Monoclonal detecting antibody	Monoclonal antibody binding (*A*)	
			Before digestion	After DNAase digestion
Whey from milk	19	Anti-DNA	1.85	0.01
		Anti-histone	0.39	0.13
DNA-binding material from milk	0.027	Anti-DNA	0.01	0.01
		Anti-histone	0.26	0.18
Total histone	0.002	Anti-DNA	0.02	0.03
		Anti-histone	0.11	0.18

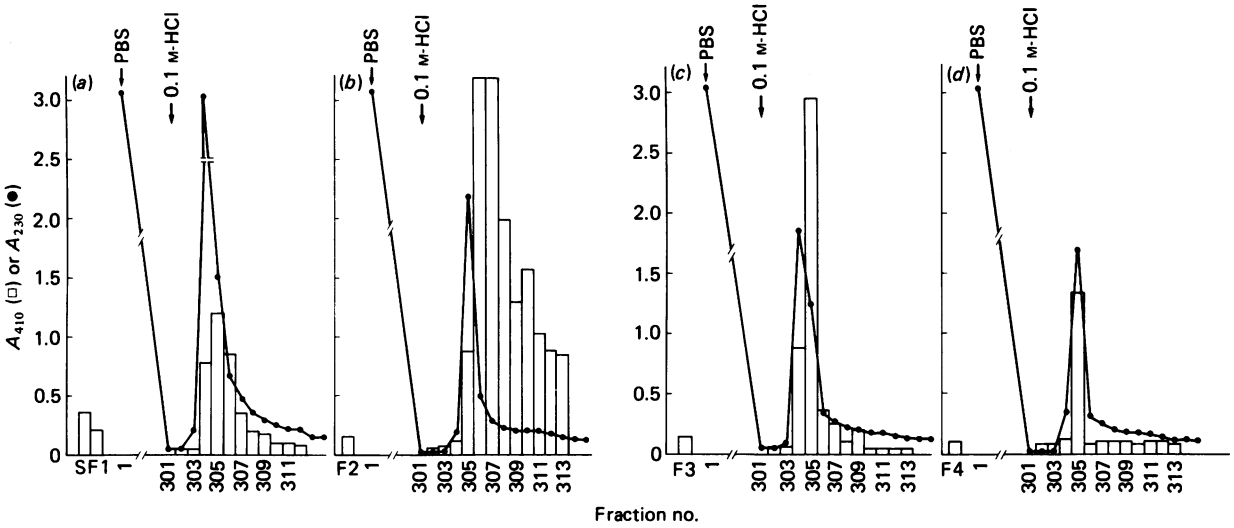

Fig. 7. DNA–cellulose affinity chromatography of proteins from FBS

A 1-litre portion of FBS was applied to a DNA–cellulose column (*a*) and elution was performed as described in the legend to Fig. 3. The unbound flow-through protein from this column (F1) was applied to a second column (*b*). Flow-through from this column (F2) was applied to a third (*c*), and sequentially a fourth (*d*), column. Fractions obtained after washing each column with PBS and eluting with HCl were analysed for total protein (●) and histone activity (□).

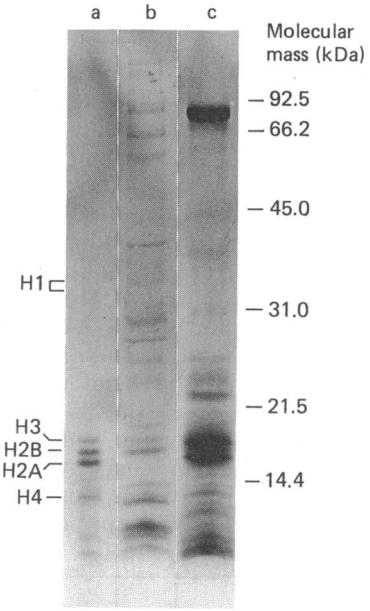

Fig. 8. SDS/polyacrylamide-gel electrophoresis of FBS DNA-binding proteins

Coomassie Blue-stained bands in the region of the gel near H3, H2B and H2A of authentic histones (lane a) were discernible in the material isolated from both FBS (lane b) and milk (lane c). Marker proteins with molecular masses in kDa are depicted.

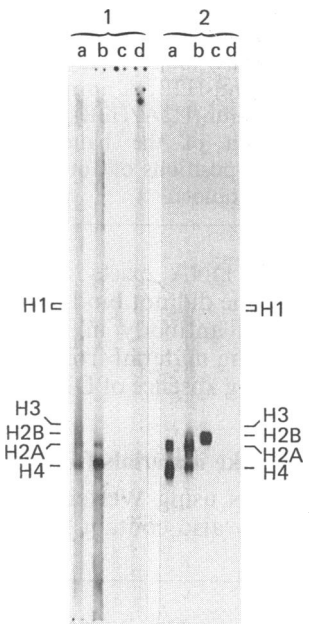

Fig. 9. Western blot of DNA-binding proteins isolated from FBS

The monoclonal antibody probes used in lanes a–d are described in the legend to Fig. 6. Proteins from milk separated by SDS/polyacrylamide-gel electrophoresis and detected with the set of mouse monoclonal anti-histone antibodies (panel 1) can be compared with histone extracted from chromatin and processed in parallel lanes (panel 2).

binding of the monoclonal anti-H2B to FBS proteins was detected (lane 1c). It appears, therefore, that only histones H2A and H4 were detected in the isolate from FBS, and H2A was partially proteolysed.

DISCUSSION

Histones were isolated from bovine milk and serum by DNA–cellulose affinity chromatography, separated by SDS/polyacrylamide-gel electrophoresis and identified with a set of monoclonal anti-histone antibodies. Over 200 macromolecules have been detected in human or bovine milk (Blanc, 1981; Anderson *et al.*, 1982), but histones have not been reported. Estimates from the immunoassay for soluble histones indicated that the recovered histone represented only 0.0003% of the total

protein in milk and 10-fold less of the serum protein content, explaining its lack of prior detection and the necessity to selectively concentrate histones from these protein-rich complex biological fluids.

DNA was readily detected in unfractionated milk by the immunoassay (Table 2), consistent with the findings of Sanguansermsri *et al.* (1974) and Jarasch *et al.* (1977). DNAase-digestion experiments indicated that at least a portion of milk histone occurred as a complex with DNA. It is possible that milk histone originated from chromatin released after mammary-epithelial-cell death or from leucocytes known to be present in post-colostrum milk (Brooker, 1978; Concha *et al.*, 1978; Blanc, 1981). Since we also detected histones in bovine serum, it is also possible that this material was transferred directly to milk in the mammary gland, as are other blood-derived macromolecules (Bruder *et al.*, 1984). The inability to detect H1 may be due to the low reactivity of this histone with the anti-histone probes (Fig. 1) or the absence of H1 in the isolate (due to its well-known sensitivity to proteolytic degradation). Proteinases are known to be present in milk (Shahani *et al.*, 1980) and serum (Erdos *et al.*, 1965), and, in fact, H2A and H2B from serum displayed the altered electrophoretic mobility characteristic of these histones in chromatin after endogenous proteolysis. H3, one of the core histones of the nucleosome (McGhee & Felsenfeld, 1980), may be present, but undetected because of a lack of the appropriate antibody probe.

Isolation of histones from serum required serial DNA–cellulose chromatography. Presumably removal of the bulk of the serum DNA-binding proteins was required before the minor histone components could favourably compete for binding to available sites on immobilized DNA. These observations are similar to those of Hoch *et al.* (1975) and Lewis & Antre (1978), who showed that serum albumin and IgG interfere with DNA–cellulose chromatography as a result of charge-dependent binding to the DNA.

Non-fat dry milk (Johnson *et al.*, 1984) and fetal bovine serum (Thomas *et al.*, 1984; Gohill *et al.*, 1985) have come under increasing use as blocking agents to minimize non-specific binding of proteins and nucleic acids to solid supports such as nitrocellulose. Spinola & Cannon (1985) compared the effect of several blocking reagents, including milk, on the identification of bacterial proteins using Western-blot technology and found different results depending on which blocking agent was used. Miskimins *et al.* (1985) recommended milk treatment to eliminate background problems with immunoblots, but also inexplicably observed enhanced reactivity of the DNA probe to histone H1 in this system. Blocking media effects may be due to contaminating nucleohistones, which may mediate the binding of the probe to reactive components on the nitrocellulose. Detection of anti-histone and anti-DNA antibodies by Western-blot techniques is particularly prone to enhanced binding artefacts when milk or fetal-bovine serum are used to the blocking reagent (S. Waga, E. M. Tan & R. L. Rubin, unpublished work).

Whether circulating histones originate from cell death and subsequent lysis or as a result of an active process of secretion (Rogers & Kawahawa, 1981; Atkinson *et al.*, 1985) may not be relevant to their immunological significance. Histones are a common target of autoantibodies in patients with drug-induced lupus (Fritzler & Tan, 1978) and systemic lupus erythematosus (Rubin, 1986*a*,*b*). The existence of histones accessible to the immune system may have consequences with respect to the origin of the anti-histone antibodies and their pathogenic potential to participate in immune-complex disease.

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